

Gene flow and population genetic structure of *Anopheles stephensi* (Diptera: Culicidae) in the areas under Enhanced Malaria Control Project (EMCP) and non-EMCP of Madhya Pradesh, Central India using microsatellite markers

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Abstract: [Aim] *Anopheles stephensi* is a major urban human malaria vector in South-East Asia contributing about twelve percent of malaria cases in India. The gene flow in *An. stephensi* belonging to the areas under Enhanced Malaria Control Project (EMCP) and non-EMCP of north-eastern regions of Madhya Pradesh (central India) was studied as initially there was a decline in malaria cases in EMCP areas due to various malaria control strategies but malaria appeared to be back-track with malaria cases increasing readily, indicating that the overall malaria risk remained stable. [Methods] The *An. stephensi* mosquitoes collected from four different areas under EMCP and non-EMCP from north eastern Madhya Pradesh (central India) were genotyped using 7 microsatellite loci for analysis of various population parameters. [Results] The markers were found to be highly polymorphic in all the populations. Not much genetic diversity was found between the two regions. Higher genetic differentiation was observed in eastern populations under EMCP ($F_{ST} = 0.0485$, $R_{ST} = 0.1112$) than northern populations under non-EMCP ($F_{ST} = 0.020$, $R_{ST} = 0.0145$) and high gene flow (12.90, 6.16, 5.06 and 2.38) was observed between EMCP and non-EMCP areas. The higher sensitivity of R_{ST} than F_{ST} indicated that the differentiation was probably caused by the mutation but not due to genetic drift. [Conclusion] The study revealed substantially high gene flow within and between EMCP and non-EMCP areas. The high gene flow combined with development of insecticide resistance seems to be a potent reason for increase in malaria cases in EMCP as well as non-EMCP areas.

Key words: *Anopheles stephensi*; microsatellite markers; gene flow; genetic differentiation; genetic drift; malaria

1 INTRODUCTION

Malaria continues to be a major public health problem worldwide and India contributes substantially to global malaria incidence (Hay *et al.*, 2010). Despite more than five decades of intensive control efforts, malaria is still a challenge to the Indian health system. *Anopheles stephensi* is an important urban malaria vector in Indian subcontinent contributing about twelve percent of malaria cases in India (Adak *et al.*, 2005) and its control has been hampered by insecticidal resistance. There is no information available about the population structure and gene flow of *An. stephensi* in India, except in Rajasthan (Vipin *et al.*,

2010a) and in Haryana (Vipin *et al.*, 2010b). The population genetics studies on *An. stephensi* using different molecular markers in different parts of the world have been reviewed by Gakhar *et al.* (2013).

Microsatellites are highly polymorphic molecular markers most widely used in population genetic studies because of their high degree of variability due to high mutation rate. Moreover, these are selectively neutral and variations are found even in populations showing low level of allozyme and mitochondrial variation.

Madhya Pradesh (MP) is the largest state situated in the central India (308 252 km²) having 50 districts with forest cover of about 31% with intense perennial malaria transmission in these areas (Mishra *et al.*, 2012). Malaria is complex in

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Madhya Pradesh because of vast tracts of forest with tribal settlement (Singh *et al.*, 2004). Enhanced Malaria Control Project (EMCP) was introduced in 1997 by National Anti-Malaria Programme (NAMP) with World Bank assistance to control the malaria in tribal/endemic areas. Initially, there was decline in malaria cases but it could not be maintained and it appeared to be back-track with malaria cases increasing readily, indicating that the overall malaria risk remains stable (Singh *et al.*, 2009). In the present study, we have therefore studied the genetic structure of *An. stephensi* in the areas under Enhanced Malaria Control Project (EMCP) and non-EMCP of central India using microsatellite DNA markers developed by Verardi *et al.* (2002). The information on *An. stephensi* between these regions should help study epidemiology. The knowledge of population genetic structure is prerequisite for implementation of adequate malaria control strategies, particularly the transgenic technology. This study should also help to understand the reasons for increase in malaria cases, particularly in highly endemic areas, where EMCP was implemented.

2 MATERIALS AND METHODS

2.1 Sample collection and identification

The adult female *An. stephensi* mosquitoes were collected from non-Endemic/non-EMCP, *i. e.*, Gwalior (26°12'46.54"N, 78°11'0.66"E), Sagar (23°49'51.66"N, 78°44'41.16"E), and Endemic/EMCP areas, *i. e.*, Umaria (23°31'31.13"N, 80°50'28.18"E) and Anuppur (23° 6'5.95"N, 81°41'52.49" E), in north-eastern region of Madhya Pradesh (Fig. 1).

Mosquitoes were collected using a hand held suction tube and torchlight from indoor resting sites. The samples were collected from two or three different areas around 10 km apart. Distance between different collection sites is given in Table 1. Intact mosquitoes were morphologically identified

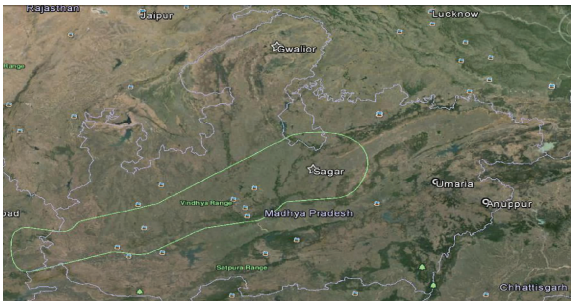


Fig. 1 Map showing collection sites of *Anopheles stephensi* mosquitoes
Star indicates non-EMCP areas and dotted circle indicates EMCP areas. Green circle represents Vindhya mountain range.

(Nagpal *et al.*, 2003) and were preserved in 95% ethanol and stored at -20°C.

Table 1 Geographic distances between population pairs of *Anopheles stephensi*

Population pairs	Distance (km)
Gwalior-Sagar	270
Gwalior-Umaria	400
Gwalior-Anuppur	495
Sagar-Umaria	216
Sagar-Anuppur	310
Umaria-Anuppur	95

2.2 Genomic DNA extraction and microsatellite genotyping of samples

Genomic DNA of 30 mosquitoes was extracted from the legs and wings of individual mosquitoes and stored at -20°C (Vipin *et al.*, 2010a, 2012b). Seven microsatellite loci developed by Verardi *et al.* (2002) were used for genotyping 30 individuals from each population of *An. stephensi*. Each pair of microsatellite markers were labeled by 6-FAM. Multiplex PCR was carried out by grouping the microsatellite primers according to their allele size range and annealing temperature (Table 2). The reaction conditions were initial heat activation at 95°C for 15 min, and denaturation at 94°C.

Table 2 List of microsatellite loci and their combinations used for multiplex PCR

Locus	Forward sequence (5' - 3')	Reverse sequence (5' - 3')	Combinations used	T _a
D8T	TCCTTCACAGCTCGGAACCTT	AGAGTATCGGCTTATGGGATG	A7 & A10	60°C
A7	CACAAATCAACCCCTTCAT	CTTCAATCACCGAAACAACAA	H2II & E12	59°C
A10	GCTGGACACGCAAGTAGG	ATAAAAGGGCACGAAACTCC	F10 & B2N	59°C
H2II	ACAATCGGTCGGAGTTCATC	AAAGCAGAGCCAAATCAAAAG	D8T	58°C
E12	ACATGCAACACCGAAACACC	CCACAGGAGGACGACTTGG		
F10	GTGGCTGCAGTACTGGGATT	GATCCAAACCGTGGTACAGC		
B2N	GCTGGGAAGTGCTACAGAGG	AAGGTTGAGTACGGACGTG		

T_a = Annealing temperature for 30 s, annealing at 57 - 60°C depending on combination used and extension at 72°C for 90 s. The numbers of cycles used were 30 - 45. Genotyping was done by ABI 3730xl Genetic Analyzer and the data were analyzed by the Genemapper 4.0 software.

2.3 Data analysis

Genetic Diversity within populations was measured by number of alleles, observed and expected heterozygosity and effective number of alleles at each locus using POPGENE version 1.32 (Yeh *et al.*, 1999) and GeneA1Ex version 6.41. Fisher exact test available on Genepop version 3.1 (Raymond and Rousset, 1995) was used to test the Hardy-Weinberg Equilibrium in each population. Exact test of genotypic disequilibrium between all the pairs of loci in all the populations was performed to know the independence of loci using Genepop version 3.1 (Raymond and Rousset, 1995). Micro-checker computer programme (Oosterhout *et al.*, 2004) was used to estimate the frequency of null alleles in all the populations at each locus. The population bottleneck was studied by Bottleneck version 1.2.02. The differentiation between populations was measured by *F*-statistics and *R*-statistics by FSTAT version 2.9.3.2 (Goudet, 1995). To investigate the relationship between genetic differentiation and geographic distance, the regression of $F_{ST} (1 - F_{ST})$ on the natural log of geographical distance was studied (Fo, 1997). The dendrogram was constructed using the Nei's genetic distances (Nei, 1978) by UPGMA method in POPGENE computer programme.

3 RESULTS

3.1 Genetic variability of *An. stephensi* populations

Genotypes of four populations (Gwalior, Sagar, Umaria and Anuppur) scored at 7 microsatellite loci indicated that these were highly polymorphic in all the populations with number of alleles ranged from 5 to 19 (Table 3). Effective number of alleles ranged from 2.00 to 12.24. A total of 87 alleles were observed in all the populations. The E12 locus was found to be the most polymorphic with 22 alleles. This locus had a maximum of 19 and 16 alleles in northern populations (non-EMCP), while 13 and 11 in eastern populations (EMCP). The observed heterozygosity varied from 0.333 to 1.000 among seven loci and the expected heterozygosity varied from 0.56 to 0.93 (Table 3). The number of alleles (N_a) and the expected heterozygosity (H_e) appeared to be slightly higher in the northern populations under non-EMCP (10.21, 0.77) than eastern population (8.4, 0.75) under EMCP of Madhya Pradesh. The four populations (Gwalior, Sagar, Umaria and Anuppur) showed more or less similar allelic distribution pattern (Fig. 2).

Table 3 Genetic diversity at 7 microsatellite loci in *Anopheles stephensi* populations

Locus		Populations			
		Northern (non-EMCP)		Eastern (EMCP)	
		Gwalior (N = 30)	Sagar (N = 14)	Umaria (N = 30)	Anuppur (N = 24)
D8T	N_a	9	6	5	7
	N_e	5. 21	3. 18	2. 75	3. 21
	H_o	0. 733	0. 566	0. 566	0. 625
	H_e	0. 82	0. 69	0. 64	0. 70
	F_{IS}	0. 109 *	0. 189 *	0. 126 *	0. 114 *
A7	N_a	11	10	9	9
	N_e	4. 42	4. 82	5. 50	5. 16
	H_o	0. 766	0. 666	1. 000	0. 625
	H_e	0. 78	0. 80	0. 83	0. 82
	F_{IS}	0. 026	0. 177 *	-0. 205	0. 245 *
A10	N_a	5	5	7	5
	N_e	2. 83	3. 04	2. 76	3. 51
	H_o	0. 600	0. 615	0. 800	0. 583
	H_e	0. 65	0. 68	0. 65	0. 73
	F_{IS}	0. 090	0. 103	-0. 236	0. 204 *
H2II	N_a	11	8	10	9
	N_e	4. 13	2. 22	5. 37	3. 72
	H_o	0. 633	0. 433	0. 333	0. 583
	H_e	0. 77	0. 56	0. 82	0. 74
	F_{IS}	0. 181	0. 229	0. 601 *	0. 223 *
E12	N_a	16	19	13	11
	N_e	12. 24	12. 24	7. 14	5. 78
	H_o	0. 700	0. 600	0. 500	0. 708
	H_e	0. 93	0. 93	0. 87	0. 84
	F_{IS}	0. 253 *	0. 361 *	0. 432 *	0. 164 *
F10	N_a	14	16	8	14
	N_e	9. 32	11. 76	3. 05	8. 53
	H_o	0. 866	0. 966	0. 466	0. 875
	H_e	0. 90	0. 93	0. 68	0. 90
	F_{IS}	0. 046 *	-0. 039	0. 321 *	0. 030
B2N	N_a	6	7	5	6
	N_e	3. 18	3. 21	3. 62	3. 27
	H_o	0. 400	0. 433	0. 800	0. 666
	H_e	0. 69	0. 70	0. 73	0. 70
	F_{IS}	0. 430 *	0. 385 *	-0. 087	0. 061
Mean	N_a	10. 28	10. 14	8. 14	8. 71
	N_e	5. 91	0. 578	4. 31	4. 74
	H_o	0. 671	0. 611	0. 638	0. 666
	H_e	0. 79	0. 75	0. 74	0. 77
SD	N_a	1. 5	2. 017	1. 079	1. 169
	N_e	1. 322	1. 633	0. 644	0. 735
	H_o	0. 056	0. 068	0. 089	0. 039
	H_e	0. 038	0. 051	0. 035	0. 028
		Average N_a = 10. 21 , Average H_e = 0. 77		Average N_a = 8. 4 , Average H_e = 0. 75	

* Significant at $P < 0.05$; N = Number of samples from each population; N_a = Number of different alleles; N_e = Effective number of alleles [Kimura and Crow, (1964)]; H_o = Observed heterozygosity; H_e = Expected heterozygosity; F_{IS} = Inbreeding coefficient calculated according to Weir and Cockerham (1984).

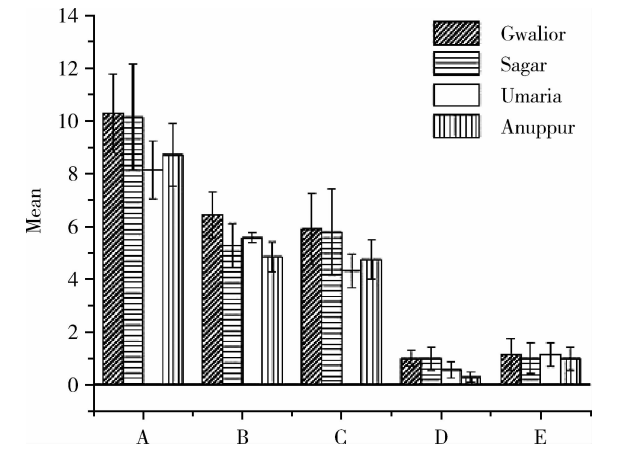


Fig. 2 Allelic distribution pattern of 7 microsatellites in *Anopheles stephensi* populations
A = Number of different alleles; B = Number of different alleles with a frequency $\geq 5\%$; C = Number of effective alleles; D = Number of unique alleles; E = Number of common alleles with a frequency $\leq 50\%$.

Three alleles 88, 173 and 87 of D8T, A10 and B2N respectively, were found to be predominating across all populations, however, allele 87 corresponding to B2N locus was found to be the most frequent and predominating (Table 4). Some unique alleles corresponding to one or other populations were also observed but their frequency was very low (data not shown). More unique alleles were present in northern population (non-EMCP) than the eastern population (EMCP).

3. 2 Hardy-Weinberg equilibrium and linkage disequilibrium observed in *An. stephensi* populations

The significant deviation from Hardy-Weinberg equilibrium (HWE) was found in 17 ($P < 0.05$) out of 28 tests within populations. Heterozygote deficiency was observed in all the cases of deviation from HWE. The loci D8T and E12 showed significant heterozygote deficiency in all the four populations. The B2N, A7, H2II and F10 showed significant deficiency in two populations whereas A10 showed deficiency only in one population (Table 1).

Significant linkage disequilibrium (LD) was observed only in 7 (8.3%) loci pair out of 84 pairs. These are D8T & A7, A7 & F10 and E12 & F10 of Sagar; D8T & A7, H2II & E12 of Umaria and A7 & H2II and D8T & B2N of Anuppur. When LD was studied locus wise-across all the populations, only one locus pair (D8T & A7) had significant LD out of 21 pairs.

3. 3 Null alleles across microsatellite loci and population bottleneck in *An. stephensi*

Null alleles were present across all the populations at 5 loci except at loci D8T and A10 (Table 5). H_{eq} (expected heterozygosity under equilibrium) based on two-phase mutation model (TPM) with fractions of multistep mutation set at

Table 4 Alleles with maximum frequencies observed in *Anopheles stephensi* populations

Populations	Alleles with maximum frequency (allelic frequency)						
	D8T	A7	A10	H2II	E12	F10	B2N
Gwalior	98(0.283)	250(0.333)	173(0.517)	127(0.433)	188, 192, 194(0.117)	178(0.200)	87(0.500)
Sagar	88(0.433)	250(0.296)	179(0.481)	127(0.650)	194(0.150)	171(0.133)	87(0.450)
Umaria	88(0.550)	252(0.300)	173(0.567)	141(0.267)	198(0.267)	179(0.533)	85, 87(0.350)
Anuppur	88(0.479)	252(0.271)	173(0.438)	127(0.438)	194(0.271)	177, 179(0.188)	87(0.435)

Table 5 Null alleles across 7 microsatellite loci in *Anopheles stephensi* populations based on overall significant excess of homozygotes with their frequencies

Locus	Populations							
	Gwalior		Sagar		Umaria		Anuppur	
D8T	No	(0.041/0.048)	No	(0.079/0.094)	No	(0.052/0.058)	No	(0.038/0.048)
A7	No	(0.004/0.004)	No	(0.078/0.086)	No	(-0.122/-0.099)	Yes	(0.112/0.126)
A10	No	(0.053/0.038)	No	(0.037/0.043)	No	(-0.197/-0.112)	No	(0.082/0.101)
H2II	No	(0.099/0.089)	No	(0.119/0.119)	Yes	(0.293/0.418)	No	(0.104/0.112)
E12	Yes	(0.115/0.134)	Yes	(0.171/0.209)	Yes	(0.207/0.264)	No	(0.070/0.077)
F10	No	(0.010/0.014)	No	(-0.030/-0.027)	Yes	(0.168/0.180)	No	(0.007/0.004)
B2N	Yes	(0.192/0.263)	Yes	(0.191/0.227)	No	(-0.053/-0.049)	No	(0.010/0.020)

The frequencies given were calculated according to the methods of Oosterhout *et al.* (2004) and Chakraborty *et al.* (1992).

30%, 20% and 10% and also based on Stepwise Mutation Model (SMM) was calculated (Cornuet and Luikart, 1996). Significant values were found under SMM in population of Sagar, Umaria and Anuppur

(Table 6). However, H_e was less as compared to H_{eq} . There was also no mode shift in frequency distribution of alleles and a normal L-shaped curve was observed for all the four populations (Fig. 3).

Table 6 Two tailed probabilities for heterozygote excess and deficiency in *Anopheles stephensi* populations based on Wilcoxon signed rank test

Population	TPM with fractions of multistep mutations set to			SMM
	30%	20%	10%	
Gwalior	0.8125	0.9375	0.9375	0.10938
Sagar	0.8125	0.9375	0.8125	0.03906 *
Jabalpur	0.2968	0.4687	0.4787	0.2968
Umaria	0.8125	1.000	0.9375	0.03906 *
Anuppur	0.8125	0.8125	0.8125	0.0156 *

* Significant at $P < 0.05$. TPM = Two phase mutation model; SMM = Stepwise mutation model.

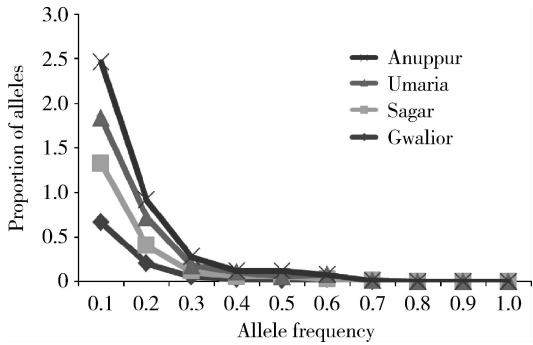


Fig. 3 Distribution of proportion of alleles in different allelic frequency classes in four populations of in *Anopheles stephensi* in Madhya Pradesh

Table 7 Genetic differentiation and gene flow (N_m) between populations of *Anopheles stephensi*

Populations	Gwalior	Sagar	Umaria	Anuppur
Gwalior	0.000	0.0145	0.0879	0.0152
Sagar	0.020 * (12.25)	0.000	0.0777	0.0567
Umaria	0.047 * (5.06)	0.095 * (2.38)	0.000	0.111
Anuppur	0.019 * (12.90)	0.039 * (6.16)	0.0485 * (4.85)	0.000

* Significant at $P < 0.05$ by bootstrapping over loci; F_{ST} values were calculated according to Weir and Cockerham, (1984); R_{ST} values were calculated according to Slatkin (1995); $N_m = 0.25(1 - F_{ST})/F_{ST}$. Figures below diagonal are $F_{ST}(N_m)$ values and above diagonal are R_{ST} values.

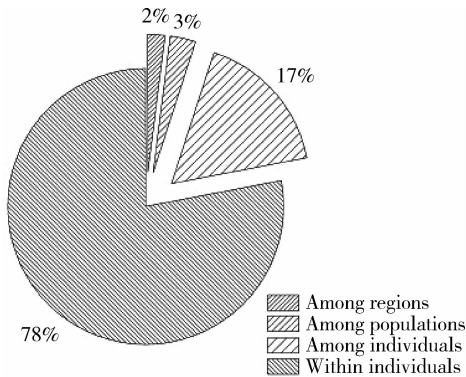


Fig. 4 Analysis of molecular variance of microsatellite loci in *Anopheles stephensi* populations based on F_{ST} values

3.5 Isolation by distance (IBD) in the *An. stephensi* populations

The analysis of isolation by distance by performing Mantel test showed a significant and

3.4 Genetic differentiation and gene flow between populations of *An. stephensi*

The level of genetic differentiation between pairs of populations was very high on eastern side, *i. e.*, in the area under EMCP ($F_{ST} = 0.0485$). The average weighted R_{ST} over loci was 0.111 (Table 7). The genetic differentiation between populations of northern region (under non-EMCP) was low as compared to eastern region. The average F_{ST} value was 0.020 and the weighted R_{ST} was 0.0145. The gene flow was found to be higher in populations under non-EMCP (12.25), *i. e.*, Gwalior and Sagar, as compared to populations under EMCP (4.85), *i. e.*, Umaria and Anuppur.

The analysis of molecular variance (AMOVA) of all the seven microsatellite loci confirmed the differentiation with the variation within individuals, among individuals within populations, among populations within groups and among groups being 78%, 17%, 3% and 2%, respectively (Fig. 4). AMOVA showed that the variation among populations within groups explained only 3% of the total variation while the variation from among individuals within populations and within individuals explained 17% and 78% of the total variation, respectively.

negative ($r = -0.4618$, $P < 0.05$) correlation between $F_{ST}/(1 - F_{ST})$ and the logarithm of distance (Fig. 5).

4 DISCUSSIONS

The genetic diversity between the populations of areas under EMCP and non-EMCP was slightly different as significant gene flow was observed between these areas. However, no previous study on population genetic structure of *An. stephensi* has been reported from Madhya Pradesh. The genetic diversity is measured with number of alleles and expected heterozygosity. The genetic diversity in northern region (non-EMCP) was slightly higher than the eastern population (EMCP). All the seven microsatellite markers used in the study were polymorphic with number of alleles at each locus

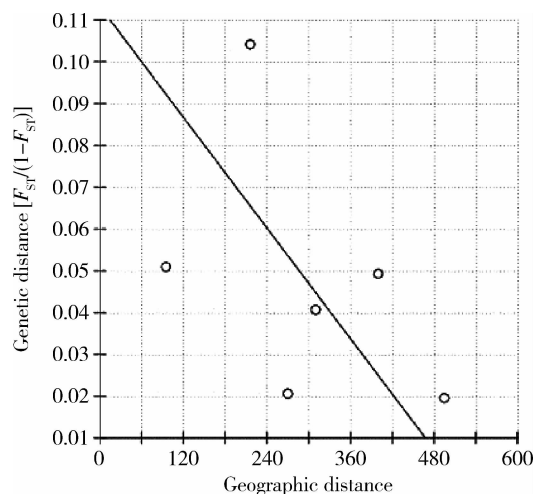


Fig. 5 Correlation between $F_{ST}/(1 - F_{ST})$ and logarithm of distance (in km) for pairwise comparison of populations of *Anopheles stephensi* at 7 microsatellite loci

ranged from 5 to 19 (Table 1). The deviations from Hardy-Weinberg Equilibrium and heterozygote deficiency were observed in all the populations studied. The heterozygote deficiency results from the wahlund effect, inbreeding or presence of null alleles (Vipin *et al.*, 2010a, 2010b). The wahlund effect or inbreeding might not be the cause of heterozygote deficiency as samples were collected from indoor resting sites only and mosquitoes belonged from a single region or two or three different sites within 5 to 10 km range. Further, only 7 significant linkage equilibrium deviations were found ruling out the possibility of wahlund effect or inbreeding. The heterozygote deficiency seems to be caused by null alleles as confirmed by their presence at 5 loci across all populations. Null alleles, *i. e.*, mutations may prevent annealing of primer resulting in either total failure of amplification or, most commonly amplification of single strand resting in scoring of genotype as homozygote (Lehmann *et al.*, 1996). No evidence of mis-scoring of stutter was detected as there was no deficiency of heterozygote with alleles differing in size by a single repeat and an excess of large heterozygote.

The significant deviation ($P < 0.05$) found under SMM in Sagar, Umaria and Anuppur populations (Table 2), appeared to be associated with H_e less than H_{eq} indicating the absence of bottleneck (Vipin *et al.*, 2010a, 2010b). In a population that experienced bottleneck recently, the mutation-drift equilibrium transiently disrupts and the heterozygosity measured at majority of loci exceeds to HWE equilibrium heterozygosity (H_{eq}) (Cornuet and Luikart, 1996). Normal L-shaped

curve was observed as there was no mode shift in frequency of allele distribution, indicating that no recent event of population bottleneck has occurred in any population.

High genetic differentiation was observed in Eastern populations (under EMCP) than Northern populations (non-EMCP). High level of gene flow was also found between EMCP and non-EMCP areas except Sagar and Umaria, where stretches of Vindhya mountain range might be acting as a geographical barrier. The genetic differentiation was probably caused by mutation and not by genetic drift as indicated by the high sensitivity of R_{ST} than F_{ST} . The isolation by distance was rejected by the negative correlation between genetic differentiation and geographic distance between sampling sites as evident by mantel's test. This indicated that neighboring populations on average are more genetically differentiated than geographically distant ones. This was further supported by the fact that significantly less genetic differentiation and high gene flow was observed between population pairs that were geographically further apart and *vice versa*. The presence of local barriers to the gene flow could be the reason for higher differentiation between nearer populations. The Vindhya mountain range might be acting as a barrier in the populations of Sagar and Umaria. In addition the vast forest cover could also affect the gene flow of urban malaria vector *An. stephensi*. The negative role of geographical distance in differentiation indicated the possibility of passive migration of *An. stephensi* which could be influenced by heavy human transport in these areas.

The UPGMA dendrogram constructed according to Nei (1978) indicated that population of Gwalior, Sagar and Anuppur are closer genetically than Umaria (Fig. 6).

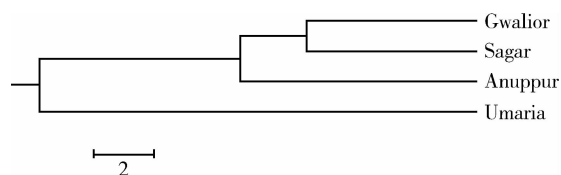


Fig. 6 UPGMA dendrogram showing genetic distance between populations of *Anopheles stephensi*

The study revealed that the genetic differentiation within eastern populations (under EMCP) was higher as compared to that the northern populations (non-EMCP). Higher gene flow was seen within northern populations than the eastern. However, significant gene flow was seen between EMCP and non-EMCP areas (Table 5), except the population of Umaria which seems to be isolated from

all other populations where less gene flow was observed. This could be ascribed to the stretches of Vindhya mountain range between these populations/regions which might act as an effective barrier in gene flow. In addition, thick forest cover in and around the Umaria might also be preventing the gene flow. Further studies are needed to understand the role of Vindhya range to prevent the gene flow and transmission of malaria. In 1997, Enhanced Malaria Control Programme (EMCP) was launched by National Vector Borne Disease Control Programme (NVBDCP) with World Bank assistance to control tribal malaria. Despite World Bank initiative several focal outbreaks swept across the state between 2003 and 2006. Though, the number of malaria infections and *P. falciparum* declined (77% and 69%, respectively) initially as a result of intensive intervention measures in districts under EMCP, malaria cases started increasing (up to 50%) in areas which are not under EMCP and where malaria was never a problem. The number of deaths also showed an alarming increase (Singh, 2006). Our study revealed a high gene flow between the EMCP and non-EMCP areas of North Eastern Madhya Pradesh which coupled with the spread of insecticide resistance could be one of the main reasons for the increase in the malaria cases, in spite of implementation of adequate malaria control strategies. The development of resistance in *An. culicifacies* mosquito to pyrethroids has already been reported in Madhya Pradesh. Pyrethroids are extensively used in malaria control programmes for indoor residual spray and for impregnation of bed nets (Mishra *et al.*, 2012). High migration rate in these areas could result in spread of insecticide resistance resulting in increase and stabilization of mosquito population.

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References

- Adak T, Singh OP, Das MK, Wattal S, Nanda N, 2005. Comparative susceptibility of three important malaria vectors *Anopheles stephensi*, *Anopheles fluviatilis* and *Anopheles sundaicus* to *Plasmodium vivax*. *J. Parasitol.*, 91(4): 79–82.
- Chakraborty R, De Andrade M, Daiger SP, Budowle B, 1992. Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Ann. Hum. Genet.*, 56(1): 45–47.
- Cornuet JM, Luikart G, 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genet.*, 144: 2001–2014.
- Gakhar SK, Sharma R, Sharma A, 2013. Population genetic structure of malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Ind. J. Experi. Biol.*, 51: 273–279.
- Goudet J, 1995. FSTAT (version. 1.2): a computer program to calculate *F*-statistics. *J. Hered.*, 86: 485–486.
- Hay SI, Okiro EA, Gething PW, Patil AP, Tatem AJ, Guerra CA, 2010. Estimating the global clinical burden of *Plasmodium falciparum* malaria in 2007. *PLoS Med.*, 7(6): e1000290.
- Kimura M, Crow JF, 1964. The number of alleles that can be maintained in a finite population. *Genet.*, 49: 725–738.
- Lehmann T, Hawley WA, Kamau L, Fonenille D, Simard F, Collins FH, 1996. Genetic differentiation of *Anopheles gambiae* populations from East and West Africa: comparison of microsatellites and allozyme loci. *Hered.*, 77: 192–200.
- Mishra AK, Chand SK, Barik TK, Dua VK, Raghavendra K, 2012. Insecticide resistance status in *Anopheles culicifacies* in Madhya Pradesh, Central India. *J. Vect. Bor. Dis.*, 49(1): 39–41.
- Nagpal BN, Srivastava A, Kalra NL, Subbarao SK, 2003. Spiracular indices in *Anopheles stephensi*: a taxonomic tool to identify ecological variants. *J. Med. Entomol.*, 40(6): 747–749.
- Nei M, 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genet.*, 89: 583–590.
- Oosterhout CV, Hutchinson WF, Wills DP, Shipley P, 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Not.*, 4(3): 535–538.
- Raymond M, Rousset F, 1995. GENEPOP version 3.1, a population genetic software for exact tests and ecumenisms. *J. Hered.*, 86: 248–249.
- Rousset F, 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genet.*, 145(4): 1219–1228.
- Singh N, 2006. Tribal Malaria, an update on changing epidemiology. In: Proceedings of National Symposium on Tribal Health, RMRCT, ICMR, Jabalpur. 10: 19–20.
- Singh N, Dash AP, Thimasarn K, 2009. Fighting malaria in Madhya Pradesh (Central India): are we losing the battle? *Mal. J.*, 8: 93.
- Singh N, Dash AP, Varun BM, Kataria OM, 2004. Tribal malaria. *ICMR Bullet.*, 34: 1–10.
- Slatkin M, 1995a. A measure of population subdivision based on microsatellite allele frequencies. *Genet.*, 139(1): 457–462.
- Slatkin M, 1995b. Hitchhiking and associative over dominance at a microsatellite locus. *Mol. Biol. Evol.*, 12(3): 473–480.
- Veradi A, Donnelly MJ, Rowlandi M, Townson H, 2002. Isolation and characterization of microsatellite loci in the mosquito *Anopheles stephensi* Liston (Diptera: Culicidae). *Mol. Ecol. Not.*, 2(4): 488–490.
- Vipin, Dube M, Gakhar SK, 2010a. Gene flow in malaria vector *Anopheles stephensi* (Diptera: Culicidae) across the Aravalli Hills in North-west India. *Entomol. Res.*, 40(2): 85–96.

Vipin, Dube M, Gakhar SK, 2010b. Genetic differentiation between three ecological variants (‘ type ’, ‘ mysorensis ’ and ‘ intermediate ’) of malaria vector *Anopheles stephensi* (Diptera: Culicidae). *Insect Sci.*, 17(4): 335 – 343.

Weir BS, Cockerham CC, 1984. Estimating *F*-statistics for the analysis of population structure. *Evol.*, 38: 1358 – 1370.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX, 1999. POPGENE, the User Friendly Software for Population Genetic Analysis, Version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

基于微卫星标记的印度中部 Madhya Pradesh 地区疟疾强化控制和非强化控制区的斯氏按蚊基因流和种群遗传结构分析

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摘要:【目的】斯氏按蚊 *Anopheles stephensi* 是亚洲东南部城市人体疟疾的主要媒介,印度 12% 的疟疾病例由其引起。本实验研究了印度中部 Madhya Pradesh 地区东北部的疟疾强化控制(EMCP)区和非强化控制(非 EMCP)区斯氏按蚊的基因流。在 EMCP 区,由于采用了各种疟疾防控措施因而疟疾病例首先降低,但是很快回升,说明总的疟疾风险维持稳定。【方法】应用 7 个微卫星位点,对印度中部 Madhya Pradesh 地区东北部的 4 个 EMCP 区和非 EMCP 区采集的斯氏按蚊进行基因分型,以分析各种群参数。【结果】发现各标记在所有种群中表现出高度的多态性。在两区间未发现很大的遗传多样性。观察到 EMCP 区的东部种群($F_{ST}=0.0485$, $R_{ST}=0.1112$)比非 EMCP 区的北部种群($F_{ST}=0.020$, $R_{ST}=0.0145$)具有较高的遗传分化,在 EMCP 区和非 EMCP 区之间观察到较高的基因流(12.90, 6.16, 5.06 和 2.38)。 R_{ST} 的灵敏度高于 F_{ST} ,说明分化可能是由于突变而非遗传漂变引起的。【结论】本研究表明,在 EMCP 区和非 EMCP 区内以及 EMCP 区和非 EMCP 区之间存在很高的基因流。基因流水平高以及抗虫性的发展似乎是 EMCP 区和非 EMCP 区疟疾病例发生增加的重要原因。

关键词: 斯氏按蚊; 微卫星标记; 基因流; 遗传分化; 遗传漂变; 疟疾

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